Effects of a Drag-Reducing Polyelectrolyte of Microscopic Linear Dimension (Separan AP-273) on Rat Hemodynamics

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Separan AP-273, a polydisperse, high molecular weight (10^3–10^7 dalton range), anionic polycrylamide, demonstrates polymer drag reduction (Toms effect) in the flows of several substances including blood. That is, under appropriate conditions, pipe flow can be increased markedly for a given pressure gradient, sometimes threefold or more, by nanomolar concentrations of linear macropolymer. The effect of intact and degraded Separan was tested in the open-chest, anesthetized rat, and left ventricular and carotid pressures, aortic blood flow (electromagnetic flow probe on ascending aorta), and the electrocardiogram were recorded. The results indicate that aortic flow, which in control animals was about half the flow reported for conscious rats, increases markedly after injection of Separan. The effect on blood pressure after an initial fall was variable, but the slope of arterial diastolic pressure was consistently steepened. Electrocardiographic changes were unremarkable except for a slow decline in heart rate. Although secondary reflexes undoubtedly influenced the results, the primary mechanism of the polymer appears to involve a reduced resistance to flow. The results are compatible with the proposal that the Toms effect, or some variant of it, is operative in vivo under favorable conditions. If this view is correct, then Separan and two other chemically different macropolyelectrolytes with similar hemodynamic effects and physical attributes, i.e., linearity and molecular lengths approaching 100 µm, might be prototypes of a novel category of cardiovascular drugs. (Circulation Research 1987;61:787–796)

Polymer drag reduction, or the Toms effect, an extraordinary hydrodynamic phenomenon little known among biomedical workers, can decrease internal resistance in liquid flow so markedly under appropriate conditions that nanomolar concentrations of certain polymers increase flow as much as threefold or more under a constant driving pressure.1,2 Although most studies have involved pipe flows of water at supercritical Reynolds numbers, the phenomenon has been demonstrated in a wide variety of fluids, including blood, and in various types of in vitro flow.

A decade ago, the first of several reports3–6 appeared which suggested that at least some drag-reducing polymers have effects in vivo that are compatible with a drag-reducing mechanism. The present report mainly describes the hemodynamic effects of Separan AP-273, a polyelectrolyte macromonomer, at a single dose of 2.0 mg/kg before and after shearing scission of the macroion into shorter fragments; the latter process is known to cause the Toms effect to disappear. The results are generally compatible with the hypothesis that drag-reducing macropolyelectrolytes of molecular lengths several times greater than the diameters of erythrocytes may increase cardiac output by a mechanism related to the Toms effect.

Materials and Methods

Preparation of Separan AP-273 Solution

Separan AP-273 (Dow Chemical Co., Midland, Mich.) is an anionic polycrylamide in neutral or alkaline solutions, with sodium as its counterion. In the molecular formula for the linear polyelectrolyte, [CH(CH(CONH2))x][CH(COONa)]y, the ratio x:y is ~7.7 The “average” molecular weight of Separan AP-273 is estimated to be ~6×10^4 daltons, but the weight ranges from under 3×10^4 to over 4×10^7 daltons. Although few macromolecules approaching the size of Separan AP-273 are more readily soluble in aqueous solutions, particular care must be exercised in preparing pharmaceutical solutions of this industrial product to avoid molecular entanglement and aggregation.

A preweighed sample of Separan AP-273 (0.2 g) was sprinkled onto the condensate formed on the inner surface of a chilled beaker. To dissolve the polymer in 100 ml 0.9% saline solution without forming gel lumps, the wetted powder was gently washed off the surface under a steady flow of saline solution from a squeeze bottle. The polymer solution was next agitated for 5 days on an orbital shaker (60–70 rpm) at ambient temperature. Large deviations from this procedure of agitation risked incomplete dispersion of the polymer or its degradation, either of which caused inconsistencies in the hemodynamic results. After thorough dissolution of the macroion, the solution was trans-
ferred to a cellulose dialysis bag (Spectrapor 2, 12,000–14,000-dalton cutoff. Spectrum Medical Industries, Inc., Los Angeles). The bag was tied about 2 cm above the solution level and immersed into 2 l dialysate containing 145 mM NaCl, 1 mM CaCl₂, and 10 mM HEPES [4-(2-hydroxy-ethyl)-1-piperazinethanesulfonic acid] buffer adjusted to pH 7.8 with NaOH. This dialysate was stirred mechanically and changed twice over a period of at least 6 days. The polymer retentate was finally diluted to 0.04% with the buffer solution, after which time it could be safely injected intravenously.

Although a 0.2% aqueous solution of Separan AP-273 is quite viscous, 46 centipoise (cP) at 120 sec⁻¹ and 25° C (Wells-Brookfield LVT cone/plate digital viscometer, Brookfield Engineering Lab., Stoughton, Mass.), inclusion of saline electrolytes decreases the viscosity to 16 cP. The viscosity is further reduced to ~7 cP by the end of dialysis.¹

Rat Hemodynamic Preparation

Sprague-Dawley female rats (n = 98), weighing 200–300 g, were anesthetized with an initial intraperitoneal dose of 40 mg/kg sodium pentobarbital solution. For experiments lasting over 1 hour, 0.2 ml of a 10-mg/ml pentobarbital solution was injected subcutaneously in the abdominal region at 15-minute intervals. This protocol kept the animal in a relatively constant and deep level of anesthesia, and the volume injected maintained constant fluid balance, verified by weighing each rat before and after the experiment. Body temperature was monitored with a rectal probe (41TD Tele-Thermometer, Yellow Springs Instrument Co., Yellow Springs, Ohio) and maintained close to 37° C with a heat lamp.

A polyethylene cannula was inserted into the trachea after tracheostomy and attached to a respirator (Harvard Apparatus rodent respirator model 680, Ealing Sci. Ltd., St. Laurent, Quebec). The lungs and heart were exposed by a midline thoracotomy, and with respiration rate held constant at 80 strokes/min, stroke volume was adjusted to optimize respiration without overinflating the lungs. A stroke volume of ~3 ml humidified air generally seemed adequate.

The pericardium was next removed, the thymus deflected, and an electromagnetic (EM) flow probe with 1.91 mm i.d. (model EP-100, Carolina Medical Instruments Inc., King, N.C.) slipped around the aorta. On placement of the aortic flow probe, the flow rate held constant at 80 strokes/min, stroke volume was adjusted to optimize respiration without overinflating the lungs. A stroke volume of ~3 ml humidified air generally seemed adequate.

The carotid artery was isolated from the vagus nerve, cannulated with a length of PE 50 tubing filled with heparinized saline, and attached to a P23GD pressure transducer (Gould Inc., Oxnard, Calif.). A 20-gauge Teflon catheter was inserted 3 to 5 mm through the apex of the heart into the chamber of the left ventricle and connected to a P231D pressure transducer. Zero reference was fixed at the level of the right atrium for both transducers. Electrocardiography electrodes were inserted subcutaneously in the right foreleg and left hind leg; a third lead connected the right hind leg to ground. A 25-gauge butterfly needle was inserted into the exposed left femoral vein and connected via polyethylene tubing to a 5.0-ml syringe containing Separan solution. A Sage 341 syringe pump (Orion Res. Inc., Cambridge, Mass.) was used to inject the polymer solution at a rate of 0.25 ml/min.

Instrumentation and Calibration

An 8-channel direct writing recorder (model 4568C, Hewlett-Packard, Waltham, Mass.) fitted with an oscilloscope monitor (model 1308A) was used for all hemodynamic recordings. Two channels (amplifier model 8805C) were assigned to LV and arterial blood pressures, respectively, with the LV pressure signal converted to a first derivative pressure reading, using a standard differentiator, and amplified on a third channel (model 8813A). Aortic blood flow was assessed with the EM probe connected to a signal coupler via a model 501 square-wave electromagnetic flowmeter (Carolina Medical Electronics, Inc.). Two channels were reserved as reference markers for peak and trough aortic blood flow (amplifier model 8809A). One channel (model 8811B) was used to record a standard lead II of the electrocardiogram.

The pressure transducers were calibrated to give a 2.5 cm deflection for 100 mm Hg pressure. The flowmeter was calibrated to determine the “probe factor” and to match flowmeter gain and probe sensitivity as recommended by the manufacturer. Heparinized blood was pumped at various flow rates through a short segment of isolated rat thoracic aorta, with its intercostal branches tied, immersed in a saline bath at ambient temperature; the pump flow was verified volumetrically. Small volumes of vehicle or Separan solution, calculated to be comparable to injection rate and volume of polymer solution administered in vivo, were injected into the calibration blood flow ~8 cm above the EM probe. There appeared to be a small (~5%) increment in probe sensitivity when Separan was used, whereas no change was detected with vehicle solution. No correction was made for this polymer effect, which presumably also occurs in vivo, nor for normal variations in hematocrit.

General Protocol for Obtaining Control Aortic Flow

On placement of the aortic flow probe, the flow usually fell 20–30% within 15 minutes and then remained quite constant over a period of at least 6 hours, providing that fluid balance was maintained. The initial fall in aortic flow seemed to be accentuated in these experiments in comparison with similar experiments in which the apex of the heart was not penetrated with a LV pressure probe. The flat segment of the flow tracing recorded during diastole was used as the zero flow reference and confirmed at the end of the experiment after cardiac standstill (saturated potassium chloride or pentobarbital overdose). In all experiments, aortic flow was initially recorded every 15 minutes, and the preparation was considered to be stable after 4 consecutive readings were within 10% of each other and without a trend. Provided that no
obvious abnormalities were present, the mean of these readings was taken as the control flow. In normalized data, the test readings were normalized against the mean value of this control.

On injection of the polymer, a transitory and dose-dependent downward shift of the zero baseline occurred, together with a widening of the instantaneous flow recording. Because the area under the original (control) baseline was subtracted from the area above the baseline, the mean flow derived by instrumental integration appeared to be altered little, decreased, or even briefly reduced to zero at very high polymer doses, despite clear evidence of ongoing flow from the pressure and ECG recordings. The baseline shift was tentatively attributed to altered probe sensitivity in the presence of polymer, and the diastolic zero segment of the flow recording was accordingly realigned to the control zero level. After such realignment, increments in flow were the same whether determined directly by instrumental integration or by planimetry of the recordings.

After completion of exploratory experiments designed to optimize the formulation of the Separan solution and the conditions of its administration, formal experiments were initiated, infusing a dose of 2.0 mg/kg Separan AP-273 in the vehicle solution described in "Materials and Methods." A total of 15 rats were studied at this polymer dosage. However, because the insertion of the LV pressure catheter resulted in a considerable but variable fall in cardiac output, the 5 studies with the lowest control aortic flows were arbitrarily excluded to avoid inclusion of technically questionable results. Inclusion of these data would increase the statistical variance but would not otherwise alter the principal findings of this study.

Statistics

The hemodynamic effects of Separan were assessed by comparing the mean of each variable after administration of the polymer with the mean obtained during the control period, using the t test for paired comparisons. Given that the injection of vehicle solution alone did not significantly alter the hemodynamic variables except transiently, comparison between control and test variables in the same animal was considered to result in a more efficient statistical analysis than a comparison of the variables in paired control and test animals. All data are described as mean ± SD unless otherwise indicated.

Results

Exploratory Experiments With Separan AP-273

Preliminary tests over a range of polymer concentrations established that injection of Separan solutions much greater than 0.04% tended to be deleterious. At a polymer concentration of 0.04%, a dose of 2.4 mg/kg was safe and hemodynamically effective, provided that injection rate did not exceed 1 ml/min × kg. In the earliest experiments, the vehicle solution consisted of 0.9% NaCl, 5 mM Na₂EDTA (ethylenediaminetetraacetate), and 6 mM CaCl₂, with the pH adjusted to 7.4.

The hemodynamic effects were variable in these experiments, but several consistent features emerged. Figure 1 shows results that are typical in all respects except for the unusually long duration of the flow increment. The most striking and consistent effect of Separan in this and later vehicle formulations was a marked widening of the instantaneous flow record, i.e., the peak rose and the zero flow (diastolic) baseline fell below the control (instrumental) zero flow level. The effect on blood pressures was quite variable, except that peak and mean aortic pressures fell initially and the slope during diastole became steeper. The initial fall in blood pressure was transient, frequently overshooting the control value 1 minute after injection ended, but the steepening of diastolic decay was sustained for approximately an hour. Heart rate generally tended to decline, rarely increasing and then only slightly and briefly.

If the results are taken at face value, high doses of Separan appeared initially to triple and occasionally even quadruple aortic flow, but this measurement was...
CONTROL  1-MIN POST-INJ.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Ventricular dP/dt</td>
<td>110/73 (87)</td>
</tr>
<tr>
<td>Carotid Blood Pressure</td>
<td>100 (mmHg)</td>
</tr>
<tr>
<td>Aortic Blood Flow</td>
<td>5.00</td>
</tr>
<tr>
<td>Electrocardiogram</td>
<td>371</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>322, 314</td>
</tr>
</tbody>
</table>

FIGURE 1. Effect of a 2.0-mg/kg dose of 0.04% Separan AP-273 solution infused intravenously in an open-chest rat after 1-hour control period of constant (±10%) aortic blood flow. Normalized flow values in B, C, and F are considered to be exaggerated because of a temporary increase in probe sensitivity at higher polymer concentrations (see text). Three hours after injection, a second and rapid injection of Separan was made. F shows typical response to a toxic dose of Separan.

The effects of Separan on electrical activity were relatively minor. Left ventricular pressure tended to be slightly elevated soon after injection of the polymer and for at least the first 30 minutes (Table 2). Surprisingly, the end-diastolic LV pressure initially rose despite the large increment in aortic flow, suggesting a marked increase in venous return. Although measurement of LV end-diastolic pressure was relatively imprecise, the suggestion of an early increase in venous return was supported by an obvious increase in atrial end-diastolic volume during this period.

Aortic blood pressure also tended to rise soon after injection of the polymer, except for end-diastolic pressure, which rose later in the experiment despite the prolongation of the diastolic period (Table 3). The most striking effects observed soon after injection of the polymer were a widening of the pulse pressure and an increase in the rate of diastolic decay followed by an elevation of the dicrotic notch. The effects on pulse pressure and diastolic decay, both variables related to arterial runoff, were greatly diminished midway through the experiment, but the effect on the dicrotic notch persisted.

The aortic blood flow and several hemodynamic variables dependent on this measurement are given in Table 4. Part of the flow increment, and thus its derivative variables, undoubtedly was associated with

FIGURE 2. Effect of a 2.0-mg/kg dose of 0.04% Separan AP-273 solution infused intravenously in an open-chest rat after a 1-hour control period of constant (±10%) aortic blood flow. Normalized flow values in B, C, and F are considered to be exaggerated because of a temporary increase in probe sensitivity (see text). Three hours after injection, a second and rapid injection of Separan was made. F shows typical response to a toxic dose of Separan.

The results obtained in 10 rats administered a 2.0 mg/kg dose of polymer are shown in Tables 1-4. Figures 2 and 3 exemplify a moderate and a particularly powerful (and prolonged) flow response to the drug, respectively. Figure 2F also shows a typical recording following additional, apparently toxic doses of Separan.

Table 1 shows the effect of Separan on heart rate, the durations of diastole and systole, and the P-R interval. With rare exceptions (e.g., see Figure 1), heart rate declined about 10% over a 2-hour period. Increases in both systolic and diastolic durations were responsible for the prolongation of the cardiac cycle, but the effect of Separan on these two variables differed. Systole was prolonged soon after administration of the polymer and then approached its control duration, whereas the diastolic duration increased slowly throughout the experiment, even after most effects of Separan had nearly disappeared. The effects of Separan on electrical activity were relatively minor.

Left ventricular pressure tended to be slightly elevated soon after injection of the polymer and for at least
TABLE 1. Effect of Separan AP-273 (2.0 mg/kg) on Heart Rate, Duration of Diastole and Systolic Ejection, and P-R Interval

<table>
<thead>
<tr>
<th>Post-injection time (min)</th>
<th>Heart rate (beats/min)</th>
<th>Duration of diastole (msec)</th>
<th>Systolic ejection duration (msec)</th>
<th>P-R interval (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>322 ± 38</td>
<td>112 ± 21</td>
<td>77 ± 11</td>
<td>44 ± 4</td>
</tr>
<tr>
<td>1</td>
<td>306 ± 41*</td>
<td>116 ± 25</td>
<td>84 ± 13</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>15</td>
<td>295 ± 33†</td>
<td>119 ± 21</td>
<td>87 ± 20*</td>
<td>48 ± 10</td>
</tr>
<tr>
<td>30</td>
<td>297 ± 37†</td>
<td>123 ± 25*</td>
<td>83 ± 18*</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>60</td>
<td>294 ± 44†</td>
<td>129 ± 27†</td>
<td>80 ± 18</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>90</td>
<td>291 ± 43†</td>
<td>130 ± 25†</td>
<td>80 ± 18</td>
<td>46 ± 6</td>
</tr>
<tr>
<td>120</td>
<td>288 ± 54*</td>
<td>138 ± 30†</td>
<td>77 ± 19</td>
<td>48 ± 8</td>
</tr>
</tbody>
</table>

Summary of results obtained in 10 rats (mean ± SD), except that 1-minute postinjection data were recorded in only 6 experiments. For the purpose of making the latter data comparable with data based on n = 10, each 1-minute mean value was adjusted by multiplying it by the mean of the 6 data values normalized against their respective controls; ±SD was adjusted accordingly. The durations of systolic ejection and diastole were estimated from the aortic pressure recording, spanning the period from end-diastole to the dicrotic notch and from notch to end-diastole, respectively. Statistical significance of all test data based on a comparison with the respective controls; ±SD was adjusted accordingly. The durations of systolic ejection and diastole were estimated from the aortic pressure recording, spanning the period from end-diastole to the dicrotic notch and from notch to end-diastole, respectively. Statistical significance of all test data based on a comparison with the control (zero postinjection time): *p<0.05, †p<0.01.

The hypothesis that the hemodynamic effects of Separan are primarily dependent on its unusual physical attributes—molecular linearity, stiffness, and extraordinary length—was tested by comparing results obtained after injection of the intact and shear degraded polymer. Polymer drag reduction in pipe flow is not manifested when macropolymers are mechanically fragmented.10-12 This test consisted of 6 experiments using the same protocol as in the previous study, except that the Separan solution was stirred for 20 hours with a magnetic Teflon rod rotating at about 1,000 rpm before injection; these experiments were terminated 1-hour postinjection.

Comparisons of the effects of mechanically degraded Separan against the effects of intact Separan, injected in equal doses and volumes, are illustrated in Figure 5. The hemodynamic response to the shortened polymer was similar to injection of an equal volume of the polymer vehicle solution alone (F.I. Polimeni and B.T. Ottenbreit, unpublished observations), including a

TABLE 2. Effect of Separan AP-273 (2.0 mg/kg) on Ventricular Blood Pressures (mm Hg)

<table>
<thead>
<tr>
<th>Post-injection time (min)</th>
<th>End-diastolic</th>
<th>Peak systolic</th>
<th>Mean systolic</th>
<th>Mean ventricular</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>122 ± 14</td>
<td>107 ± 13</td>
<td>45 ± 7</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>118 ± 31</td>
<td>113 ± 35</td>
<td>51 ± 14</td>
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</tr>
<tr>
<td>15</td>
<td>134 ± 21</td>
<td>124 ± 26</td>
<td>53 ± 11</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>129 ± 20</td>
<td>120 ± 19</td>
<td>50 ± 9*</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>124 ± 19</td>
<td>113 ± 19</td>
<td>46 ± 8</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>125 ± 12</td>
<td>117 ± 15</td>
<td>48 ± 7</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>120 ± 18</td>
<td>115 ± 20</td>
<td>45 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

Determined simultaneously with Table 1. *p<0.05.

The rate constant (k) of exponential decay was calculated as k = -ln(P_{up}/P_{eq})/t_{up}. *p<0.05, †p<0.01, ‡p<0.001.

TABLE 3. Effect of Separan AP-273 (2.0 mg/kg) on Aortic Blood Pressure

<table>
<thead>
<tr>
<th>Post-injection time (min)</th>
<th>Peak systolic</th>
<th>Diastolic decay rate and rate constant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dicrotic notch</td>
<td>Linear (mm Hg/sec)</td>
</tr>
<tr>
<td>0</td>
<td>113 ± 12</td>
<td>164 ± 39</td>
</tr>
<tr>
<td>1</td>
<td>118 ± 23</td>
<td>381 ± 101†</td>
</tr>
<tr>
<td>15</td>
<td>128 ± 21</td>
<td>328 ± 74*</td>
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<tr>
<td>30</td>
<td>126 ± 18</td>
<td>276 ± 67*</td>
</tr>
<tr>
<td>60</td>
<td>123 ± 17</td>
<td>233 ± 71†</td>
</tr>
<tr>
<td>90</td>
<td>123 ± 10</td>
<td>210 ± 59*</td>
</tr>
<tr>
<td>120</td>
<td>121 ± 16</td>
<td>203 ± 79</td>
</tr>
</tbody>
</table>

Determined simultaneously with Table 1. The decline of the aortic blood pressure during diastole was evaluated, assuming a linear or exponential diastolic decay. The linear decay rate (ΔP_{a}/Δt_{a}) was calculated by dividing the difference between end-systolic (P_{es}) and end-diastolic (P_{eq}) pressures by the duration of diastole (t_{a}). i.e., ΔP_{a}/Δt_{a}=(P_{es}-P_{eq})/t_{a}.

The rate constant (k) of exponential decay was calculated as k = -ln(P_{up}/P_{eq})/t_{up}.

*p<0.05, †p<0.01, ‡p<0.001.
**Table 4. Effect of Separan AP-273 (2.0 mg/kg) on Apparent Aortic Blood Flow and Some Derivative Variables**

<table>
<thead>
<tr>
<th>Post-injection time (min)</th>
<th>Aortic blood flow (ml/min-kg)</th>
<th>Stroke volume (ml/beat-kg)</th>
<th>Mean ejection rate (ml/sec-kg)</th>
<th>Cardiac external work (J/min-kg)</th>
<th>Total peripheral resistance (mm Hg-min/ml-kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>105 ±16</td>
<td>0.34 ±0.07</td>
<td>4.3 ±0.8</td>
<td>1.5 ±0.3</td>
<td>0.90 ±0.13</td>
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<tr>
<td>1</td>
<td>235 ±37‡</td>
<td>0.79 ±0.15†</td>
<td>9.5 ±2.3‡</td>
<td>3.8 ±1.8*</td>
<td>0.42 ±0.06†</td>
</tr>
<tr>
<td>15</td>
<td>233 ±63‡</td>
<td>0.81 ±0.28†</td>
<td>9.4 ±3.0t</td>
<td>3.8 ±1.0†</td>
<td>0.48 ±0.16†</td>
</tr>
<tr>
<td>30</td>
<td>201 ±63‡</td>
<td>0.71 ±0.30†</td>
<td>8.6 ±3.6†</td>
<td>3.3 ±1.0‡</td>
<td>0.58 ±0.22†</td>
</tr>
<tr>
<td>60</td>
<td>158 ±46†</td>
<td>0.56 ±0.23†</td>
<td>7.3 ±3.6*</td>
<td>2.6 ±1.1*</td>
<td>0.72 ±0.27*</td>
</tr>
<tr>
<td>90</td>
<td>149 ±53*</td>
<td>0.54 ±0.26*</td>
<td>6.8 ±3.4*</td>
<td>2.5 ±1.0*</td>
<td>0.82 ±0.37†</td>
</tr>
<tr>
<td>120</td>
<td>130 ±48</td>
<td>0.48 ±0.22</td>
<td>6.3 ±3.3</td>
<td>2.2 ±1.0</td>
<td>0.92 ±0.39†</td>
</tr>
</tbody>
</table>

Determined simultaneously with Table 1. These data do not take into account the coronary component of cardiac output. Since all values in this table depend on measurements of the aortic flow probe, which may have increased its sensitivity in the presence of polymer, all derived values must be considered to be nominal with their accuracy increasing at later postinjection times. Thus, these data are included only to indicate the trend and approximate magnitude of the Separan-induced changes. The stroke volume (SV), mean ejection rate (MER), cardiac work (CW), and total peripheral resistance (TPR) were calculated from the following equations: SV = CI/HR, MER = SV/t,, CW = CI x PS x PLV x t^, and TPR = Pa/CI, where CI is cardiac index, HR is heart rate, Ps is mean ventricular pressure during systole, PLV is mean LV pressure, Pa is mean aortic pressure, ts is duration of systole, and tc is duration of cardiac cycle.

* p<0.05,
† p<0.01,
‡ p<0.001.

**Discussion**

The addition of certain stiff, linear polymers to turbulent flows greatly reduces flow friction, a phenomenon known as "polymer drag reduction" or the "Toms effect." On addition of minute molal concentrations of such polymers, twofold or threefold flow increments are common when the driving pressures are held constant. Alternatively, when the polymers are added to a constant flow system, the pressure gradient falls by a comparable magnitude. Most of the experimental data published refer to turbulent flows, but a substantial body of evidence has established that polymer drag reduction is operative in various types of flows, other than turbulent, which might be described as disturbed. The Toms effect has never been observed in a linear laminar flow, although several reports indicate that this effect can occur in nonlinear flows generally considered to be laminar. The precise mechanism of polymer drag reduction remains elusive, but it is generally accepted that the effect involves a stabilizing laminarization of flow structure. Indeed, such laminarization is readily visualized by injecting dyes or other markers into turbulent flows observed through transparent conduits. It must be emphasized that polymer drag reduction in pipe flow depends on a reduction of flow friction associated with force vectors other than those parallel to the flow axis; thus, it is unrelated to viscous drag reduction. In a fluid such as blood, these vectors would include the oblique and tumbling motions of...
Figure 5. Hemodynamic effects of a 2.0 mg/kg dose of Separan AP-273 before ("intact polymer," ○, n = 10 except that n = 6 at time 1 minute) and after ("sheared polymer," ●, n = 6) mechanical shearing of the polymer. All polymer postinjection data were normalized with respect to their respective control values before statistical analysis. The same protocol was used for both groups except that the "sheared polymer" group were administered Separan after the polymer solution was subject to 20 hours of stirring at ~1,000 rpm. Error bars represent mean ± SEM.

eythrocytes. It is noteworthy that polymer drag reduction also has been observed (mechanism unknown) in aqueous flow through a column of sand,17 where interstitial dimensions were comparable to those of the microcirculatory vasculature.

All polymers displaying the Toms effect have two fundamental features in common: linearity of primary molecular structure and lengths approaching or exceeding 1 μm. The effect is favored by molecular stiffness and, for aqueous flows, an ionic or at least
polar character. The Toms effect is lost when the polymer molecule is fragmented into shorter segments or reconstituted in a nonlinear conformation. Four polymers, each characterized by molecular lengths approaching or exceeding 100 μm, are known to reduce drag in blood flow through pipes: a deoxyribonucleic acid obtained from the calf thymus, an okra polysaccharide extract, the poly(ethylene oxide) Polyox WSR-301, and the polyacrylamide Separan AP-30. The latter macromolecule is essentially the same as Separan AP-273, except that it has an average molecular weight of ~4 × 10^6 instead of ~6 × 10^6 daltons. No polymer shorter than the diameters of several erythrocytes seems to have reduced drag in blood flow.

Mostardi et al. appear to have been the first to report a study on the effect of a drag-reducing agent in vivo, showing evidence that Separan AP-30 dampened flow disturbances distal to an aortic constriction using hot-film anemometry. A similar finding was reported recently in a study utilizing a 20-MHz pulsed Doppler velocimeter, in which Separan AP-30 and AP-273 were found to reduce poststenotic turbulence significantly in the canine carotid artery. On the basis of the anemometric findings, Mostardi postulated that Separan AP-30 might inhibit atherogenesis because atherosclerotic lesions tend to form at vascular sites of disturbed flow. This hypothesis was supported by visual examination of the aortae of rabbits and white Carneau pigeons on high cholesterol diets. The finding in rabbits was recently confirmed by a morphometric assessment of atherogenesis in the presence and absence of Separan AP-30.

Shortly after Castro showed that an aqueous extract of okra reduced drag in turbulent water and blood flows in vitro, Polimeni et al. isolated a polysaccharide, identifying it as a linear rhamnogalactogalacturonan (RGGu), that markedly enhanced rat cardiac output. Parenthetically, Benjamin et al. had earlier reported the unexpected recovery of some dogs subjected to experimental hemorrhage and then administered a crude okra extract solution, one that undoubtedly included RGGu, in a search for an abundant plasma expander. Because the isolation and purification of native RGGu in amounts sufficient for broad pharmacologic testing in large animals did not prove feasible, other drag-reducing polymers with similar hemodynamic effects were sought. Two such polymers that appear to augment cardiac output in rats, the anionic polyacrylamide Separan AP-273 and the poly(ethylene oxide) Polyox WSRN-60K, show hemodynamic effects similar to those demonstrated by RGGu despite the chemical dissimilarities of the three macromolecules. It follows, therefore, that the effect of these polymers on in vivo flow is most likely due to the physical attributes they have in common, i.e., linearity and great length. This suggestion is further supported by 1) the demonstration that the hemodynamic effects of Separan nearly disappear when the polymer macromolecule is shortened, 2) the failure to observe any vasoactive responses to Separan in a variety of isolated arteries and veins, and 3) a lack of inotropic effect on isolated ventricular muscle by Separan at concentrations comparable to those that are hemodynamically effective in vivo.

The most consistent hemodynamic finding with Separan AP-273, common to the effects of RGGu and Polyox, was a marked increase in the amplitude of the aortic flow recording. The control flow measured with the EM probe was lower than values reported by several other investigators applying a variety of techniques to the rat, including some using EM probes, but comparable to results obtained with similar probes by Pfeffer and Frohlich, Tobia et al., and Walsh et al. Measurements of cardiac index in rats extend over a nearly threefold range of values in the literature, partly because of differences inherent in the various methodologies used (e.g., see Walsh et al.), with EM measurements tending to be in the lower range. However, EM measurements of relative changes of flow are considered to be quite reliable.

Anesthetics are known to influence strongly several hemodynamic variables, including blood flow. Pentobarbital in particular has consistently been found to depress the cardiac index markedly compared with indexes in both conscious rats and rats anesthetized with other commonly used anesthetics. It is noteworthy that in the present study, the cardiac index reached at the peak of the polymer effect approached values recorded with EM probes in unanesthetized rats, suggesting that the increment of flow after administration of the polymer compensates in large measure for flow reduction associated with anesthesia, thoracotomy, and penetration of a relatively large LV catheter through the myocardium.

Accurate quantification of the aortic flow after injection of the polymer is problematic because the diastolic baseline shift is apparently associated with an increased sensitivity of the electromagnetic flow probe. This baseline shift is also observed with Polyox but minimally with RGGu and then only at high polymer concentrations. In vitro calibration with blood flows through isolated aortae, measured volumetrically, shows that the increase in probe sensitivity exaggerates the recorded flow by less than an order of magnitude compared with the increment observed in vivo. However, this result cannot be extrapolated quantitatively with confidence to the in vivo condition where the increase in probe sensitivity might be intensified. It is known that alteration of the flow velocity profile could account for an increased probe sensitivity, but the profile does not appear to be altered by Separan, even downstream from an arterial stenosis. If an increase in blood flow by Separan is indeed related to the Toms effect (i.e., a stabilization or laminarization of flow by an alignment of linear macroions tending to corral erythrocytes into laminar arrays) then it might be expected that such an effect would be accentuated in pulsatile flow through the geometrically complex vasculature in vivo, compared with a steady flow through a segment of isolated aorta whose luminal irregularity is mainly confined to its end connections.
If it can be assumed that the baseline shift represents a heightened probe sensitivity, then the results suggest that the accuracy of the flowmeter after baseline recovery is comparable to that during the preinjection control period. As a corollary to this argument, although postinjection flow readings obtained before baseline recovery are exaggerated, the true values are likely to be between the nominal recorded values and the highest value recorded after baseline recovery. In general, such recovery was observed when the flow increment was equal to or less than about 60% of the control value. For this reason, although normalized flow values, i.e., postinjection flows relative to preinjection flows, above 1.60 are considered to be nominal, their actual values presumably lie between the nominal reading and 1.60. It is also assumed that this deviation from a true value was greatest at the highest blood concentration of polymer, and the deviation disappeared as the diastolic zero-flow reference line approached the instrumental zero baseline asymptotically. The decay of the Separan effect on total peripheral resistance and slope of arterial pressure during diastole (Figure 4) suggests that the polymer was degraded or eliminated with a half-life of about 30–40 minutes.

If polymer drag reduction occurs in vivo, its immediate effect presumably would be to diminish the internal resistance of blood flow as manifested by a transient fall in pressure and augmentation of flow (see Figures 2 and 3 and Tables 3 and 4). The latter appears to occur within the first minute after injection of the polymer unless the unlikely assumption is made that the flow probe is so grossly in error in the presence of polymer unless the unl ikely assumption is made that the internal resistance of blood flow as manifested by a transient fall in pressure and augmentation of flow (see Figures 2 and 3 and Tables 3 and 4). The latter appears to occur within the first minute after injection of the polymer. Since there is no reason to believe that three chemically dissimilar macromolecule drag-reducing agents, a polyacrylamide, a polysaccharide, and a poly(ethylene oxide), are likely to reduce in vivo flow resistance by vasodilation, as verified experimentally in the case of Separan AP-273, it follows that some physical mechanism is likely to be responsible for this effect.

One possible mechanism of Separan action that probably can be excluded is hypoviscosity secondary to hemodilution. Injection of a "control" solution would be expected to cause the same volume displacement as an equal volume of polymer solution, but neither the vehicle solution alone nor the vehicle solution containing sheared polymer (present experiments) enhances aortic flow other than slightly and transiently. Thus, the enhancement of flow by the polymer solution cannot be explained by a hematocrit-dependent hypoviscosity effect unless an expansion of plasma volume is postulated via a Starling transcapillary mechanism. Such a mechanism seems unlikely because intravenous injections of Separan into rabbits on a high-cholesterol diet do not appear to alter hematocrit. That drag-reducing polymers induce transcapillary fluid movements cannot be entirely excluded because the rabbit study was a long-term one, and it now appears that both Separan and Polyox are potent diuretics acutely. However, diuresis is unlikely to be associated with hemodilution in the absence of excess fluid intake, unless there is a shift of fluid from the interstitium into the plasma compartment. Such a shift is unlikely given the anomalous osmotic effect of Separan.

As one might expect when any pharmacologic prototype based on an unfamiliar mechanism is introduced, more questions arise than are answered. The question of toxicity, for example, has not been addressed at this stage of research, other than to note that no deleterious effects are obvious in intact rats and rabbits over a period exceeding 6 months after single or multidose injections of Separan. A transient hematuria is frequently observed soon after injection of the polymer, but it does not appear to compromise renal function.

Examination of the recordings and data suggests that whatever the primary mechanism of Separan, secondary reflexes are likely to influence the results. Attempts to study the effects of Separan on larger animals (dogs and pigs) have resulted in mixed results in contrast to the easily repeatable findings in rats. In large animals, particularly those in excellent hemodynamic status, Separan usually had little (<25% aortic flow increment) or no effect. In other animals, particularly those in cardiogenic or hemorrhagic shock, hemodynamic responses were sometimes striking. Clearly, many more studies will be required for the elucidation of the hemodynamic response to polymer drag-reducing agents. Nonetheless, the present results, particularly when considered together with other available information, suggest that linear polymers with lengths of the order of 100 μm have unusual effects in the circulation with obvious therapeutic potential.

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References


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